Locking up Sisters Ensures Daughters Get a Fair Deal

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Aneuploidy – an increase or decrease in the number or composition of one or more chromosomes – is a common cause of birth defects in humans. Some babies with aneuploid cells, such as those suffering from Down's Syndrome (trisomy 21) or Turner's Syndrome (XO karyotype), mature into adults with only mild to moderate handicaps. The majority, however, are afflicted by such severe developmental abnormalities *in utero* that they die before or shortly after birth.

Aneuploidy-associated genetic imbalances lead to abnormal cellular functions through the production of either excess levels of protein (as occurs in Down's Syndrome), or insufficient levels (as occurs in Turner's Syndrome). The end result of these imbalances is perturbation of normal development. Most foetuses with genetic imbalances involving chromosomes other than the X or 21 abort spontaneously, as they typically alter the expression of a larger number of genes and perturb an increased number of cellular and biochemical processes.

The majority of aneuploidy-associated birth defects are thought to result from errors in chromosome segregation during gametogenesis or in the early cell divisions following fertilisation. In recent years, prenatal screening has had a major impact on reducing the number of affected children born with such overt karyotypic abnormalities. Nevertheless, we still know relatively little of how these errors in chromosome segregation arise in the first place.

The molecular mechanisms that ensure chromosome segregation occurs correctly have only recently started coming into focus. A greater understanding of the molecular details of how chromosome segregation is regulated can only improve our chances of developing more sophisticated screening and prevention techniques for birth defects caused by chromosome abnormalities.

Sister chromatid cohesion

Sister chromatid cohesion is one of several carefully regulated cellular mechanisms critical for ensuring a single copy of each chromosome gets partitioned into each daughter cell. Normally, newly replicated chromosomes (sister chromatids) become co-joined immediately following DNA synthesis in S phase. This linkage is normally maintained until a cell has entered the M phase of the cell cycle. Only then, after chromosomes have become highly condensed, a spindle has been assembled, and each pair of co-joined chromatids is attached via their kinetochores to opposite poles of the spindle, is sister chromatid cohesion dissolved (**Fig. 1**). This robust mechanism, which is common to all eukaryotic species, ensures each daughter cell receives an identical copy of each and every chromosome. However, errors in either the establishment or release of chromatid cohesion can cause chromosomes to be unequally distributed between the daughter cells.

The cohesin complex

For many years researchers have postulated the existence of a molecular glue that holds newly replicated chromatids together until the cell is ready to partition each chromatid into its daughter cells. In the last decade, the components of this so-called glue have been identified and their mechanisms of action studied in some detail. At the molecular level, these components act more like a lock than glue, as described below.

The primary mediator of sister-chromatid cohesion is a four-subunit protein complex, called cohesin (**Fig. 1C**). Cohesin forms the physical linkage that joins sister chromatids during S and G2 phases of the cell cycle, and is destroyed at the metaphase to anaphase transition. Cohesin-homologous genes have been found in all eukaryotic organisms examined to date, suggesting that this complex arose early in eukaryotic evolution. A combination of genetic and biochemical studies in budding yeast (*Saccharomyces cerevisiae*), fruitfly (*Drosophila melanogaster*), African clawed frog (*Xenopus laevis*), and human and mouse cells have revealed that cohesin is required for both the establishment and maintenance of

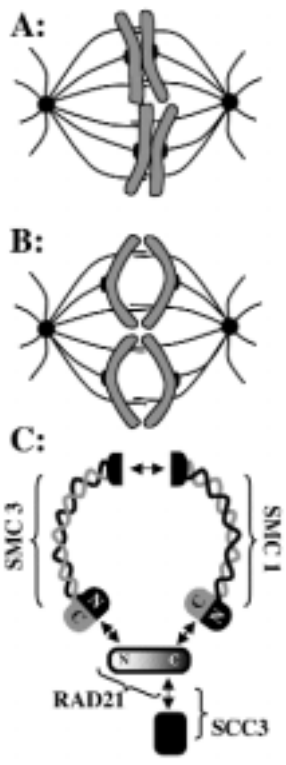

Fig. 1. Sister-chromatid cohesion is mediated by the cohesin complex.
A. Cohesion between sister chromatids is established in S phase and maintained until chromatid pairs are under tension, having nucleated microtubules from opposite poles of the spindle.
B. Simultaneous dissolution of cohesion on all chromosomes allows a single chromatid from each pair to move to each pole of the spindle.
C. Model of the cohesin complex. Double-headed arrows indicate known subunit interactions demonstrated in ref. 2.

sister chromatid cohesion.

In budding yeast, cohesin is composed of the products of four genes (1). These are called Smc1p, Smc3p, Scc1p/Mcd1p/ Rad21p and Scc3p/IrrI. As is common in many fields, the naming of proteins is not always consistent or sensible. To minimise confusion, I will henceforth refer to these four proteins, irrespective of the species or experimental system under discussion, as SMC1, SMC3, RAD21 and SCC3.

The SMC1 and SMC3 components of cohesin are members of the Structural Maintenance of Chromosomes protein family. These proteins have globular N- and C-termini, which closely associate with one another (2). The termini are linked to a central globular hinge domain by long antiparallel coiled-coils (**Fig. 1C**). The structures of the other two components (RAD21 and SCC3) are much less certain, as no crystal structures have been solved to date and primary protein sequence provides few clues as to their molecular structure or biochemical function.

SMC-family proteins are not exclusively involved in mediating sister chromatid cohesion. In higher eukaryotes, there are at least seven evolutionarily conserved SMC proteins (3). In addition to cohesin, other SMC-containing multi-protein complexes are required for chromosome condensation, dosage compensation and DNA repair. In all cases thus far examined, the non-SMC components of these complexes show no overt sequence similarity to one another. This suggests that early in the eukaryotic lineage a series of SMC gene duplications allowed the evolution of specialist SMC-containing complexes to facilitate alterations in higher order chromatin structure, with complex-specific subunits co-evolving to restrict or otherwise regulate the activities of the SMC subunits.

Several models have been postulated over the last few years to explain the relationship between the SMC and non-SMC components of cohesin and how they interact to establish and then release sister chromatid cohesion. The current model is presented in **Fig. 1C,** in which cohesin acts to establish and maintain sister-chromatid cohesion by forming a closed ring, perhaps encircling both DNA strands and thereby preventing the two sister strands of DNA from separating once the complex has been loaded and activated (2).

In many ways, cohesin has both biochemical and structural attributes similar to a padlock (**Fig. 1C** and **2**). The two SMC proteins form a heterodimer, interacting with one another via their central hinge domains to form the clasp of the lock. RAD21 forms the body of the lock, with its N-terminus binding both the N- and C-termini of SMC3, and its C-terminus binding both the N- and C-termini of SMC1. SCC3 only interacts with the C-terminus of RAD21, perhaps acting as a key that inserts into the body of the lock. Although this model can account for much of the data, many aspects of the regulation of cohesin in metazoans (described below) are still unaccounted for.

Cohesin dynamics in yeast

The cell-cycle dynamics of cohesin, as elucidated from studies in *S. cerevisiae*, are shown in **Fig. 2**. In *S. cerevisiae*, cohesin is absent from G1 cells, is synthesised in late G1 and S phase, and must be loaded onto chromatin during or shortly after DNA replication in order to effect cohesion (4). Loading of cohesin is achieved with the help of two other proteins, SCC2 and SCC4 (5). ECO1 and PDS5 are another two proteins that act on cohesin to lock the newly replicated DNA strands firmly together following DNA replication (6). In this way, sister chromatid cohesion is established at discrete chromosomal sites, which in yeast are located approximately every 10 kilobases (7). Interestingly, preventing cohesin from effecting chromatid cohesion during S phase cannot be compensated for by the later addition of cohesin in G2 (4).

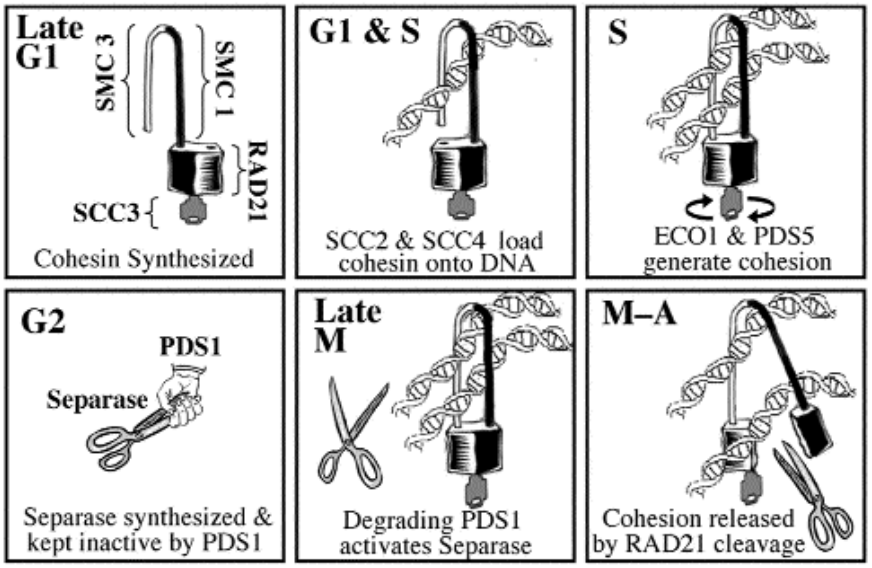

Fig. 2. Model of cohesin function in *S. cerevisiae*.
Cohesin is synthesised in late G1. Loading of cohesin onto chromosomes requires the action of both SCC2 & SCC4. During or immediately after DNA replication, cohesion is established with the assistance of ECO1 & PDS5. In G2, separase is synthesised but kept inactive by its inhibitor, PDS1. Immediately prior to the onset of anaphase, PDS1 is degraded, activating separase and initiating the loss of sister chromatid cohesion by cleaving the RAD21 component of cohesin. Cohesin is then degraded by the APC/C prior to G1.

Cohesin remains on chromatin throughout G2 and until M phase. Just prior to the onset of anaphase, the cohesin lock is broken open by site-specific cleavage of the RAD21 subunit. Severing the RAD21 linkage between the two SMC components allows sister chromatids to separate and move to opposite poles of the spindle. RAD21 cleavage is regulated by the activities of two non-chromosomal proteins, ESP1 (separase) and PDS1 (securin) (8). PDS1 associates with ESP1 in interphase, keeping it inactive until

metaphase. Anaphase Promoting Complex/Cyclosome (APC/C) mediated ubiquitination and degradation of PDS1 just prior to anaphase frees and activates ESP1, inducing proteolytic cleavage of RAD21, dissociation of RAD21 from chromatin and the triggering of sister chromatid separation (9). Following sister separation the cohesin complex is then degraded by the APC/C.

Cohesin dynamics in metazoans

At present, our understanding of cohesin function in higher species is less advanced than for yeast. Although any features seem completely conserved, several important differences have been observed between cohesin dynamics in yeast and higher species (some of which depicted in **Fig. 3**). These include:

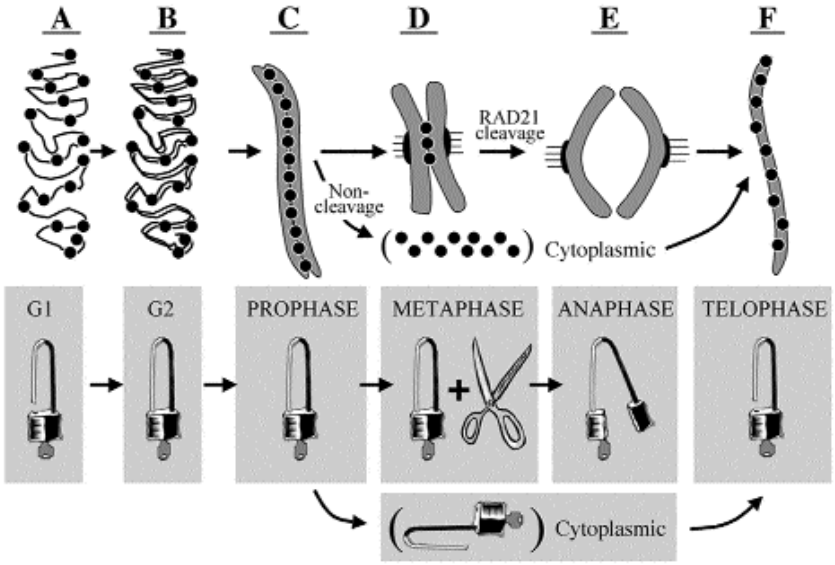

Fig. 3. Cohesin dynamics in metazoa.
A. In G1, cohesin is localised to chromatin.
B. Following S phase, cohesion is established via the locking mechanism of cohesin.
C. In prophase, as chromosomes condense, the vast majority of cohesin dissociates from chromatin and becomes cytoplasmic without RAD21 being proteolytically cleaved.
D. A small pool of centromere-proximal cohesin remains on chromosomes until the onset of anaphase, which is triggered by the activation of separase.
E. Cleavage of RAD21 allows sister chromatids to be pulled to opposite poles of the spindle.
F. In telophase, the cytoplasmic pool of cohesin reassociates with the decondensing chromatin.

(i) Intracellular cell cycle dynamics show that in vertebrates and *Drosophila*, the bulk of cohesin dissociates from chromatin in prophase, well before sister chromatids separate (10,11). Although a minor centromere-associated pool of cohesin remains on chromosomes until the onset of anaphase, cohesion along the arms is still maintained until the onset of anaphase (10, 12).

(ii) Prophase dissociation of the bulk of cohesin from chromosomes is associated with altered phosphorylation of SCC3 and not proteolytic cleavage of RAD21 (11), whereas separation of sister chromatids at the metaphase-anaphase transition requires the cleavage of the RAD21 component of the centromere-associated pool of cohesin by separase (12). Non-chromosomally located RAD21 is protected from separase cleavage by an as yet unknown mechanism.

(iii) Metazoan cohesin reassociates with chromatin in telophase and is present throughout G1 phase, whereas in yeast cohesin is completely degraded in M and is absent from G1 phase cells (1, 10).

(iv) Vertebrate and *Drosophila* cells contain two distinct classes of mitotic cohesins containing alternative SCC3-related subunits (cohesin^{SA1} or cohesin^{SA2}) (13). The significance of these two distinct types of mitotic cohesins is not yet clearly established, as studies to date have failed to show any overt differences in their chromosomal distribution at any stage of the cell cycle.

(v) A fifth as yet unidentified cohesin component has been shown to be associated with cohesin^{SA1}-containing complexes (11).

(vi) Genome project data reveals that *C. elegans* and humans have two separate RAD21 homologues and *Arabadopsis* has at least 3, suggesting that higher species may have evolved several specialist cohesin complexes reflecting additional complexity of function.

Although many features of cohesin structure and function are well conserved between higher and lower species (14), there appears to be greater complexity in cohesin activity and regulation in metazoan species, as evidenced by more intricate cell-cycle dynamics and alternative subunit variations compared to yeast. Exploration of the regulatory and functional basis of these differences is an ongoing area of research of great interest, both in my lab here at the Comparative Genomics Centre and internationally.

Cohesin function and birth defects

Does altered cohesin activity contribute to birth defects in humans? At present we don't know, chiefly because of the difficulty in accessing relevant human material. In lower species, only cohesin loaded and locked onto chromosomes during S phase can effect chromatid cohesion. Ectopic cohesin introduced to cells outside of S phase is incapable of effecting cohesion between sister chromatids (4). If this observation holds true for mammals, then this could help explain the maternal age effect, in which the chances of a woman having a baby with an aneuploid-associated birth defect increases as she gets older.

While a female human foetus is still *in utero*, cells in her developing ovary undergo pre-meiotic S phase and shortly after arrest in meiosis I. These cells remain arrested in

meiosis I for decades until they are reactivated, one each month, during follicle maturation following puberty. This raises the possibility that human cohesin, synthesised and loaded onto pre-meiotic chromosomes during embryonic development, has to remain functional for several decades without being replaced by newly synthesised protein. If this proves to be so, then birth defects caused by chromosome segregation errors, which escalate in frequency after the age of 35, could simply be the result of the cumulative attrition of embryonically-synthesised cohesin. In other words, with time the padlock might just rust enough to allow the chromosomes to fall apart.

Further studies on the function and regulation of cohesin will no doubt yield insights into these and other clinically important issues where failure to lock up sister chromatids stops daughter cells from getting a fair deal.

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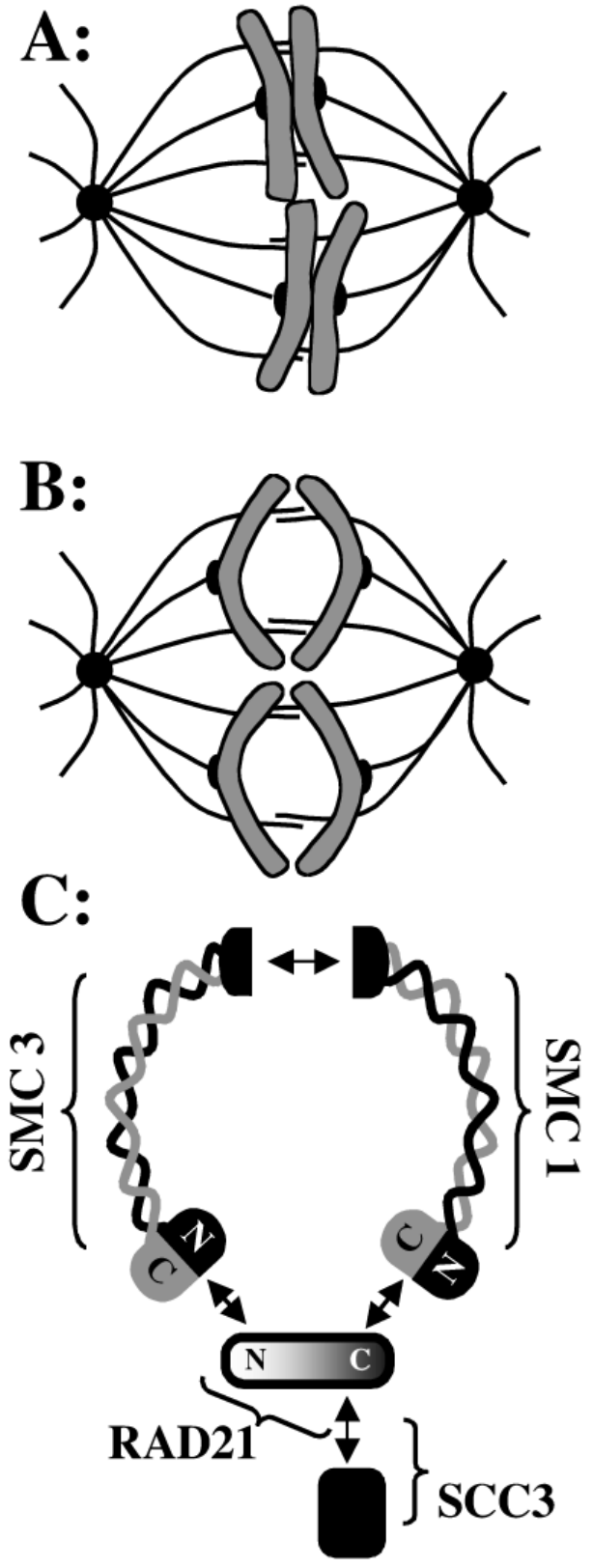

Fig. 1

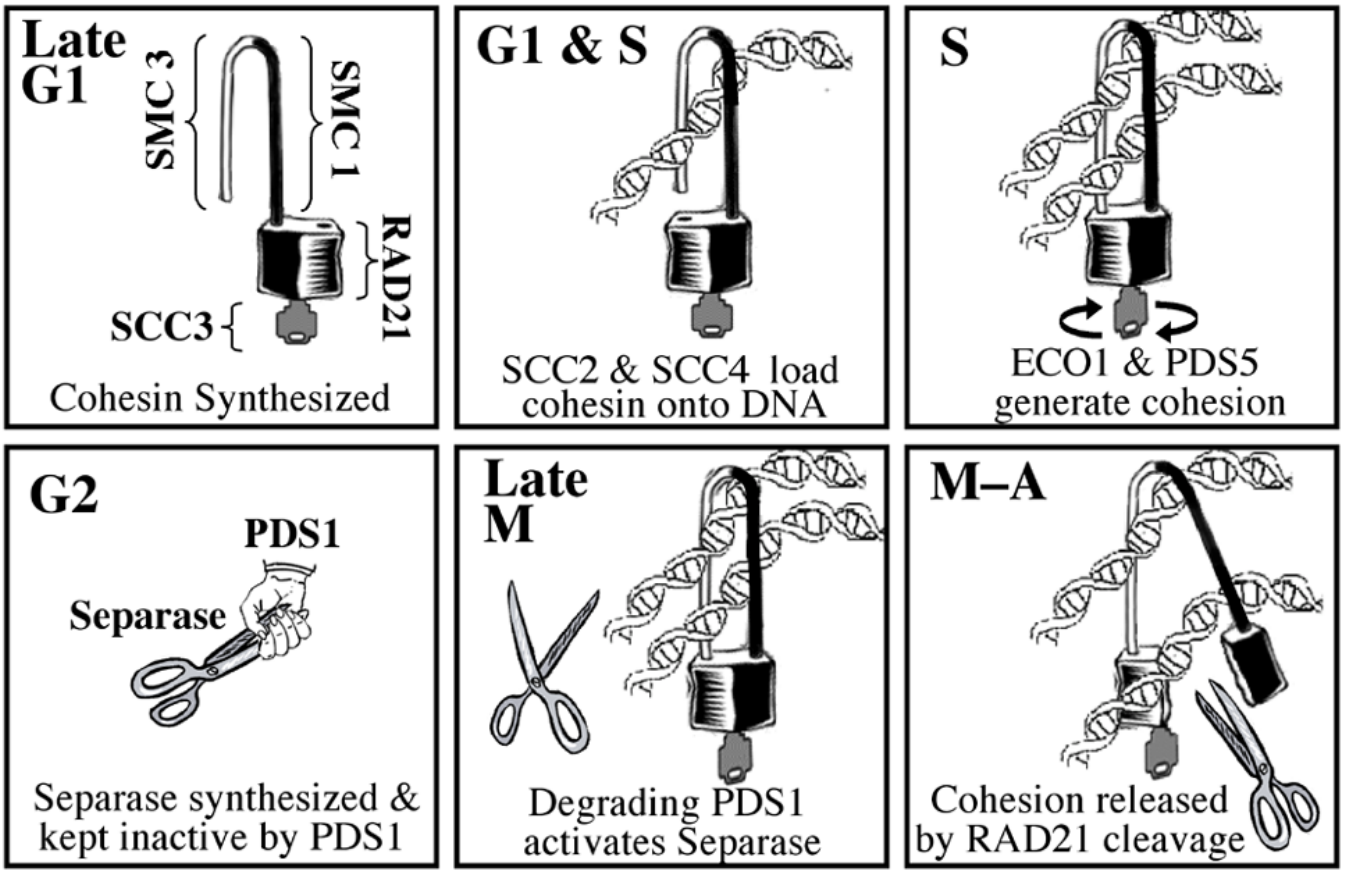
Late G1
SMC 3
SMC 1
RAD21
SCC3
Cohesin Synthesized
G1 & S
SCC2 & SCC4 load cohesin onto DNA
S
ECO1 & PDS5 generate cohesion
G2
PDS1
Separase
Separase synthesized & kept inactive by PDS1
Late M
Degrading PDS1 activates Separase
M–A
Cohesion released by RAD21 cleavage

Fig. 2

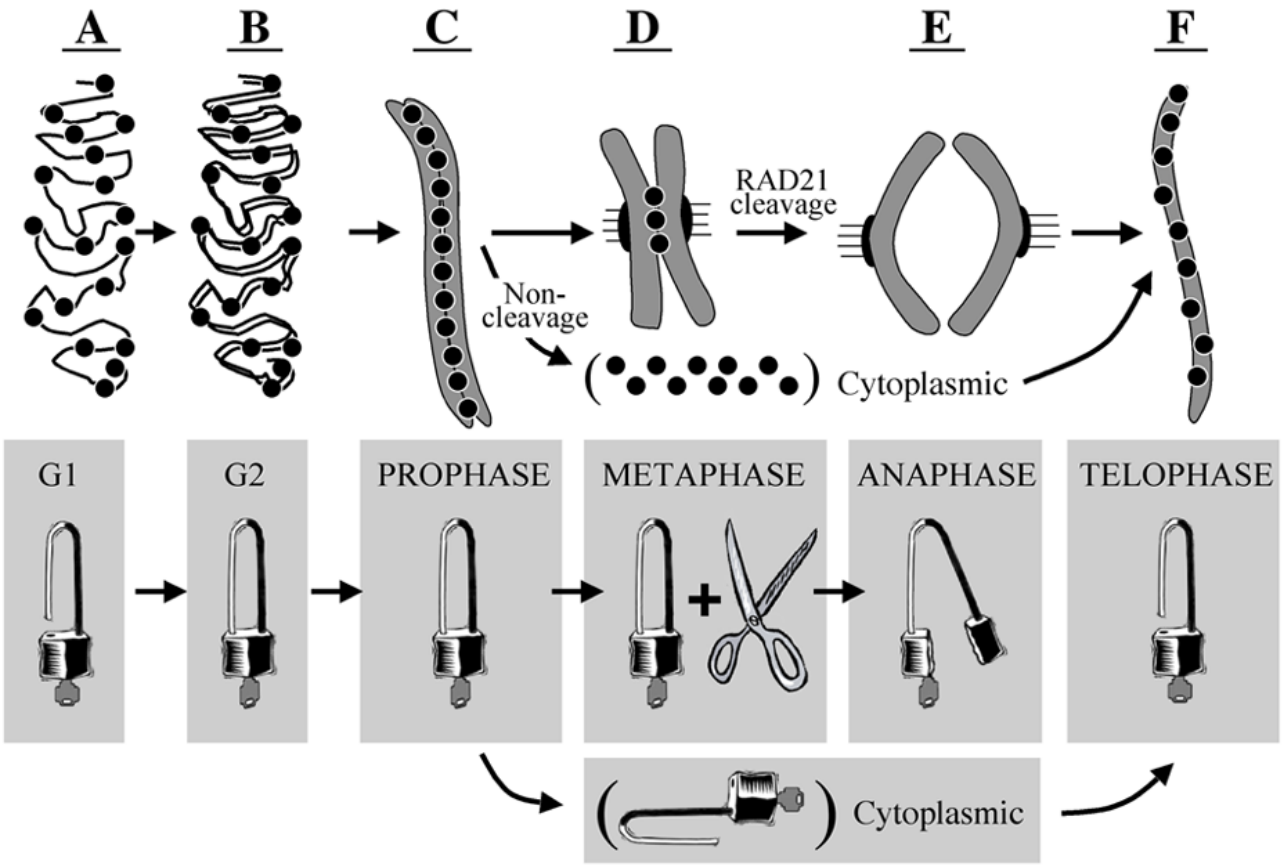
A
B
C
D
E
F
RAD21
cleavage
Non-
cleavage
Cytoplasmic
G1
G2
PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
Cytoplasmic

Fig. 3